

# Effects of Crystalloid and Colloid Resuscitation on Hemorrhage-Induced Vascular Hyporesponsiveness to Norepinephrine in the Rat

Liang-Ming Liu, MD, John A. Ward, PhD, and Michael A. Dubick, PhD

**Background:** We have shown previously that hemorrhagic hypotension is associated with a progressive development of vascular hyporeactivity to norepinephrine (NE). The present study investigated whether select crystalloid or colloid resuscitation fluids would ameliorate this effect.

**Method:** Anesthetized male rats were hemorrhaged to and maintained at a mean arterial pressure (MAP) of 50 mm Hg for 60 minutes. Rats (n = 7 per group) were then resuscitated with lactated Ringer's (LR), 7.5% hypertonic saline (HS) for 1 hour followed by LR (HS-LR), Hespan, or Hextend to restore and maintain MAP to 70 mm Hg over 4 hours. Additional hemorrhaged groups were resuscitated with LR to the baseline MAP (LR-BL) or received no resuscitation. A sham hemorrhage group served as controls. The re-

sponses of MAP and the blood flow of the superior mesenteric, celiac, left renal, and left femoral arteries to NE (3  $\mu$ g/kg administered intravenously) were measured at BL (prehemorrhage); at the end of the hypotensive period; and at 1, 2, and 4 hours after resuscitation.

**Results:** Hemorrhagic hypotension significantly ( $p < 0.01$ ) reduced the NE-induced pressor response in MAP and significantly reduced the contractile responses (reflected by the reduction of blood flow after NE administration) of the four arteries to NE. Hespan and Hextend infusion improved the NE response of MAP and the contractile responses of the observed arteries to NE significantly better than LR, HS-LR, or LR-BL. The colloids improved the vascular contractile responses to NE in the superior mesenteric and left femoral arteries and the pressor

response of MAP to NE, to 80% to 90% of their basal response level compared with 40% to 60% with the crystalloid fluids ( $p < 0.05$ ). LR-BL infusion resulted in hemodilution, with no added benefit to vascular responsiveness.

**Conclusion:** These data suggest that hypotensive resuscitation to 70 mm Hg with colloids was better than crystalloids in improving vascular responsiveness to the pressor effects of NE and required smaller volumes. Normotensive resuscitation with LR was not better than hypotensive resuscitation. Not all vasculatures improved equally after fluid resuscitation.

**Key Words:** Shock, Hemorrhagic, Hypovolemic, Vascular reactivity, Different vascular bed resuscitation, Fluid, Lactated Ringer's, Hypertonic saline, Hespan, Hextend, Rat.

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**T**raumatic hemorrhagic shock, a common clinical syndrome, is often seen in both civilian and military casualties. Both in vitro and in vivo studies, in our laboratory and others, have demonstrated that after severe trauma or shock, including hemorrhagic, endotoxic, and septic shock,

vascular reactivity to vasoconstrictors and vasodilators is greatly reduced and appears to be implicated in the occurrence, development, and outcome of shock.<sup>1–9</sup> Reduced vascular reactivity may also interfere with the therapy of shock, especially with the application of vasoactive agents.<sup>10</sup>

Fluid resuscitation is a common and very important treatment modality for many kinds of circulatory shock, especially for traumatic hemorrhagic shock. Many crystalloids and colloids have been used clinically or experimentally for resuscitation of hemorrhage,<sup>11–18</sup> but it is unknown whether these fluids would be beneficial, *in vivo*, in ameliorating the vascular hyporesponsiveness observed after hemorrhagic shock. In an *in vitro* study with aortic rings, Wang et al.<sup>19</sup> observed that endothelial cell dysfunction induced by hemorrhage persisted after fluid resuscitation with lactated Ringer's (LR).

The controversial study by Bickell et al.<sup>20</sup> suggested that limiting fluid resuscitation in patients with penetrating torso injuries may be beneficial. In addition, studies in experimental animals have suggested that allowing permissive hypotension in models of uncontrolled hemorrhage improved outcome compared with resuscitation to the baseline mean arterial pressure (MAP).<sup>21,22</sup> This concept of permissive hypotension is also attractive for managing casualties in military

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situations.<sup>23</sup> Thus, the objectives of the present study were to determine whether hypotensive fluid resuscitation (to a MAP of 70 mm Hg) would improve the decreased vascular reactivity to norepinephrine induced by hemorrhagic shock, whether the improvement would be better with colloids than crystalloids, and whether the improvement would be similar in different vascular beds. The fluids investigated in this study included crystalloids, such as LR solution as the standard of care, and 7.5% hypertonic saline (HS) and the colloids Hespan (6% hetastarch in normal saline) and Hextend (6% hetastarch solution containing balanced electrolytes; BioTime, Inc., Berkeley, CA). The observed blood vessels included the celiac (CA) and left renal arteries (LRA), representing supplies to solid organs; the superior mesenteric artery (SMA), mainly supplying a hollow organ; and the left femoral artery (LFA), for skeletal muscle. The methodology used to determine vascular responsiveness *in vivo* followed well-established protocols that used changes in MAP to estimate vascular responsiveness to a vasoconstrictor.<sup>8,24</sup>

## MATERIALS AND METHODS

### Instrumentation

This study was approved by the Research Council and by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research. All procedures adhered to the *Guide for the Care and Use of Laboratory Animals* established by the National Research Council. Male Sprague-Dawley rats, weighing  $413 \pm 31$  g, were fasted 12 hours but allowed water *ad libitum* before the experiment. On the day of experiment, rats were first anesthetized with 2% to 3% isoflurane and the right femoral artery and vein and right carotid artery were catheterized with polyethylene tubing (outer diameter, 0.965 mm; inner diameter, 0.58 mm) for monitoring the MAP, for administering norepinephrine (NE) and fluid, and for bleeding as previously described.<sup>7</sup> Within these cannulae, the tubing was filled with normal saline containing 30 U/mL of heparin to prevent clot formation and all attempts were made to minimize the amount of heparin that entered the body. The body temperature of the rats was maintained at 37°C with a heating pad.

A laparotomy was performed and the SMA, CA, and LRA were located and isolated, and fat and connective tissue were carefully cleaned off the vessels for proper acoustic coupling as described.<sup>7</sup> A 0.7 V-series flow probe (Transonic Systems Inc., Ithaca, NY) was mounted around the SMA, whereas around the CA and LRA were mounted 0.5 V-series probes. To optimize recordings over the entire experiment period, the vessels were nestled in the bottom of the V reflector, lubricating jelly was placed in the probe's acoustic window as couplant, and the flow probes were maintained with a proper stand. The LFA was then exposed, isolated, and a 0.5 V-series flow probe placed. All probes were connected to a Transonic flowmeter (Transonic Systems Inc., Ithaca, NY) for monitoring the arterial blood flows. After instrumentation, rats were allowed to equilibrate for 20 to 30 minutes.

The isoflurane was turned off and rats were anesthetized with urethane (900 mg/kg administered intraperitoneally) for the rest of the experiment.

### Experimental Protocol

Rats were randomized into seven groups: sham hemorrhage ( $n = 7$ ), hemorrhage ( $n = 7$ ), hemorrhage plus LR infusion to a MAP of 70 mm Hg (LR,  $n = 7$ ), hemorrhage plus 7.5% HS plus LR infusion to a MAP of 70 mm Hg (HS-LR,  $n = 7$ ), hemorrhage plus Hespan infusion to a MAP of 70 mm Hg (Hespan,  $n = 7$ ), hemorrhage plus Hextend infusion to a MAP of 70 mm Hg (Hextend,  $n = 7$ ), and hemorrhage plus LR infusion to the baseline MAP (LR-BL,  $n = 7$ ). The latter group was included to reflect standard resuscitation practice with crystalloids. After baseline response measurements, 42 rats were hemorrhaged rapidly (within 10 minutes) from the right carotid artery catheter until the MAP dropped to 50 mm Hg, and it was maintained at this level for 60 minutes. Our previous studies indicated that a hemorrhage of approximately 20 mL/kg was required to reduce MAP to this level.<sup>7</sup> Then, the LR group was infused with LR as needed to maintain MAP at 70 mm Hg for 4 hours. The HS-LR group was infused with HS to maintain MAP at 70 mm Hg over the first hour and then infused with LR over the remaining 3 hours. The Hespan and Hextend groups were infused with Hespan or Hextend, respectively, to maintain MAP at 70 mm Hg for 4 hours. The LR-BL group was infused with LR to maintain MAP at the baseline level for 4 hours. All fluids were administered with an infusion pump (Model AS 50, Baxter Co., Deerfield, IL). The hemorrhage group received no fluid infusion. No shed blood was reinfused in any group. Seven rats, operated on but not hemorrhaged, served as the control group (sham hemorrhage). MAP; blood flow of the SMA, CA, LRA, and LFA; and their responses to a vasoconstrictor (NE, 3  $\mu$ g/kg bolus intravenous injection)<sup>8,24</sup> were observed at baseline; at the end of the hypotensive period (0 h); and at 1, 2, and 4 hours after fluid infusion. MAP was monitored with a DigiMed blood pressure analyzer (MicroMed, Inc., Lexington, KY). Blood flow was measured with a Transonic T206 flowmeter (Transonic System Inc., Ithaca, NY), and data were captured with a DATAQ DI-220 data acquisition system and displayed with WINDAQ software (DATAQ Instruments, Akron, OH). The maximum increase in MAP after NE infusion generally reflected the systemic vascular reactivity.<sup>8,24-26</sup> Similarly, the maximal decrease in blood flow of each artery after NE administration was taken to reflect the contractile response to NE, and vascular reactivity was calculated as the relative change of blood flow before and after NE administration on the basis of the following equation: The relative change in blood flow after NE =  $(A - B)/A$ , where A = blood flow before NE administration and B = the lowest blood flow after NE administration. For each artery, the result of this calculation at baseline was assigned a value of 100%. The relative

change in blood flow at subsequent experimental times was expressed as a percentage of the baseline change.

At the end of the experimental period, the infusion volume of each fluid in each group was recorded, and the surviving rats were killed with Sleepaway (2 mL) administered through the femoral vein catheter. In addition, blood samples of animals taken at baseline and at the end of the experiment were used for determination of hematocrit (Hct) and blood  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  according to standard clinical chemistry techniques.

### Statistical Analysis

All data are presented as mean  $\pm$  SD of the number of observations. Vascular reactivity was defined as the maximum difference in MAP or blood flow in response to each injection of NE compared with pre-NE injection, and the vascular reactivity at each time point was calculated as a percentage of the response observed at baseline. Statistical differences of the changes in MAP, blood flow, and their responses to NE were assessed by a three-factor analysis of variance (treatment, time point, and vascular bed), followed by post hoc Tukey tests. The fluid infusion volume, Hct, and blood electrolytes were assessed by one-factor or two-factor (time and treatment) analysis of variance, as appropriate. The comparisons of primary interest included hemorrhage group versus sham hemorrhage group, LR and HS-LR group versus hemorrhage group, HS-LR versus LR group, Hespan and Hextend group versus LR or HS-LR group, and LR-BL group versus LR or HS-LR group. A value of  $p < 0.05$  was considered significant.

## RESULTS

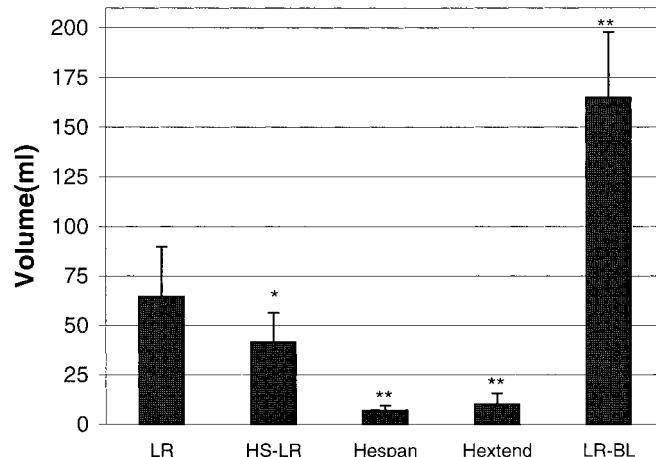
Animals in the hemorrhage group without fluid infusion survived an average of  $100 \pm 26$  minutes. All other animals survived the entire 4 hours after the end of the hypotensive period.

### Volume of Fluid Infused

A total  $64.5 \pm 25.2$  mL of LR was needed to maintain MAP at 70 mm Hg for 4 hours (Fig. 1). Infusing HS over the first hour ( $2.16 \pm 0.47$  mL, equivalent to  $5.11$  mL/kg) significantly reduced the total volume of LR ( $38.2 \pm 14.5$  mL) ( $p < 0.05$ ) required over the remainder of the experiment. The infusion volumes of Hespan and Hextend required were less than 10 mL, markedly less than the volume of LR needed. Normotensive resuscitation with LR (LR-BL group) required almost three times as much LR as in the LR group (Fig. 1).

### Changes in Hct and Serum Electrolytes

Basal Hct was similar in all groups (Fig. 2). Hct at the end of the experiment was significantly lower in all fluid resuscitation groups than in the unresuscitated hemorrhage group or in their own group at baseline. Hct in HS-LR, Hespan, and Hextend groups was reduced to 28.7%, 29.2%,



**Fig. 1.** Infusion volume of each fluid after hemorrhagic shock in rats. LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg for 4 hours; HS-LR, 7.5% hypertonic saline infusion for 1 hour followed by lactated Ringer's solution to a MAP of 70 mm Hg,  $41.4 \pm 14.9$  mL in this group includes  $2.16 \pm 0.47$  mL of hypertonic saline; Hespan, Hespan infusion to a MAP of 70 mm Hg for 4 hours; Hextend, Hextend infusion to a MAP of 70 mm Hg for 4 hours; LR-BL, LR infusion to baseline MAP for 4 hours. Data expressed as mean  $\pm$  SD for seven rats per group. \* $p < 0.05$ , \*\* $p < 0.01$  as compared with the LR group.

and 31.5%, respectively. Hct was significantly higher in the Hespan and Hextend groups as compared with the LR group. The lowest Hct was in the LR-BL group and was significantly lower than Hct in the LR group (Fig. 2).

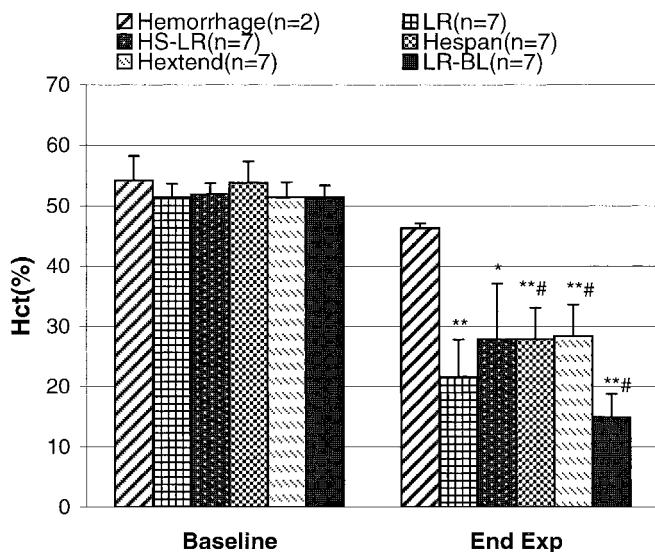
Baseline serum electrolytes were similar in all groups (Table 1). In general, after hemorrhage, with or without fluid resuscitation, serum  $\text{K}^+$  and  $\text{Cl}^-$  were significantly elevated compared with baseline. Serum  $\text{Na}^+$  was increased 4 mEq/L in the HS-LR group at the end of the experiment ( $p < 0.01$  from baseline) but was unchanged in the other groups as compared with their corresponding baseline value.

### Changes in MAP

As expected, MAP in the sham hemorrhage group remained unchanged throughout the experiment (Table 2). The decrease in MAP in the unresuscitated hemorrhage group progressively worsened during the course of the experiment. All fluid resuscitation groups were maintained at a MAP of 70 mm Hg or at baseline MAP through continuous fluid infusion according to the experimental protocol (Table 2).

### Changes in the Pressor Response to NE on MAP

Hemorrhagic hypotension at 50 mm Hg for 60 minutes without return of shed blood or fluid infusion induced a marked loss of overall vascular reactivity (Fig. 3). The pressor response to NE on MAP was reduced to 44.1%, 18.4%, and 6.84% of their basal response at the end of hypotensive period and 1 and 2 hours after the end of hypotensive period,



**Fig. 2.** Hematocrit (Hct) at baseline and at the end of the experimental period. LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg for 4 hours; HS-LR, 7.5% hypertonic saline infusion for 1 hour followed by lactated Ringer's solution to a MAP of 70 mm Hg for 3 hours; Hespan, Hespan infusion to a MAP of 70 mm Hg for 4 hours; Hextend, Hextend infusion to a MAP of 70 mm Hg for 4 hours; LR-BL, LR infusion to baseline a MAP for 4 hours. Data expressed as mean  $\pm$  SD. \* $p$   $<$  0.05, \*\* $p$   $<$  0.01 as compared with the hemorrhage group; # $p$   $<$  0.05 as compared with the LR group.

respectively (Fig. 3). LR or HS-LR infusion to a MAP of 70 mm Hg improved the pressor response of MAP to NE approximately 25% (Fig. 3). The effect of HS infusion was better than LR alone at the 1-hour time point, but the continued infusion of LR in the HS-LR group did not improve vascular responsiveness over that observed with LR alone. Hespan and Hextend infusion markedly restored the decreased pressor response of MAP to NE, and their effects were significantly superior to LR or HS-LR infusion at 2 and 4 hours. LR infusion to return MAP to the baseline level (LR-BL group) significantly improved vascular reactivity compared with the LR group at 1 hour, but prolonged infu-

sion resulted in deteriorated vascular reactivity at 4 hours. The pressor response of MAP to NE in the sham hemorrhage group did not change significantly during the whole experiment (Fig. 3).

### Changes in Blood Flow of the Observed Arteries

In the sham hemorrhage group, the blood flow did not change significantly during the experimental period in any artery, whereas in the hemorrhage group, blood flow decreased markedly in all arteries (Table 3). LR infusion to maintain a MAP of 70 mm Hg significantly increased the blood flow in all arteries observed ( $p$   $<$  0.05– $p$   $<$  0.01) compared with the hemorrhage group. HS infusion over 1 hour resulted in lower blood flows in comparison with the LR group. At 2 and 4 hours, blood flow in the four arteries was similar in the LR and HS-LR groups (Table 3). The improvement in arterial blood flow after Hespan and Hextend infusion was similar to that in the LR group at 1 hour, but at 4 hours, these agents improved blood flow better than LR in the LFA and LRA. Blood flows in all arteries generally were higher after Hextend than Hespan infusion, but the differences were not statistically significant. LR infusion to the baseline MAP resulted in the highest blood flows in all arteries, and these levels were significantly higher than the LR, HS-LR, and sham hemorrhage groups (Table 3).

### Response of Blood Flow to NE

The relative responses of blood flows to NE in the four observed arteries, as reflected by the decrease in blood flow after NE administration and taken as an estimate of vascular reactivity, were similar in the sham hemorrhage group during the experimental period (Fig. 4). In contrast, hemorrhagic hypotension caused a marked loss in this response to NE in the observed arteries at the end of the hypotensive period. For example, the responses of the SMA, CA, LFA, and LRA were reduced to 49.3%, 47.4%, 36.3%, and 47.7% of their baseline response, respectively. At 2 hours in the unresuscitated hemorrhage group, over 90% of the responsiveness to NE in the SMA and LRA was lost, whereas it was lost entirely in the LFA and CA (Fig. 4). LR or HS-LR infusion to a MAP of 70

**Table 1** Na, K, and Cl (mEq/L) before Hemorrhage and after Fluid Infusion after Hemorrhagic Shock

	Baseline			End of Experiment		
	Na	K	Cl	Na	K	Cl
Hemorrhage group (n = 2)	140.5 $\pm$ 2.12	4.0 $\pm$ 0.14	104.5 $\pm$ 0.71	141.5 $\pm$ 2.12	6.9 $\pm$ 1.13*	107.5 $\pm$ 0.71
LR (n = 7)	139.5 $\pm$ 0.71	4.1 $\pm$ 0.14	104.0 $\pm$ 1.41	137.0 $\pm$ 1.41	5.9 $\pm$ 0.07*	111.5 $\pm$ 0.71*
HS-LR (n = 7)	139.8 $\pm$ 1.57	3.9 $\pm$ 0.44	104.3 $\pm$ 1.11	143.7 $\pm$ 1.70**	6.2 $\pm$ 1.17**	118.1 $\pm$ 2.96**
Hespan (n = 7)	138.4 $\pm$ 2.38	4.0 $\pm$ 0.48	101.7 $\pm$ 2.24	138.0 $\pm$ 1.57	5.6 $\pm$ 0.66**	108.1 $\pm$ 4.60**
Hextend (n = 7)	138.4 $\pm$ 1.51	4.1 $\pm$ 0.47	103.7 $\pm$ 1.11	136.0 $\pm$ 4.00	6.4 $\pm$ 0.85**	108.4 $\pm$ 2.63**
LR-BL (n = 7)	139.4 $\pm$ 2.25	4.1 $\pm$ 0.39	103.1 $\pm$ 1.72	138.8 $\pm$ 5.69	4.5 $\pm$ 0.43 <sup>②</sup>	112.8 $\pm$ 4.22**

LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg; HS-LR, 7.5% hypertonic saline infusion for 1 h followed by lactated Ringer's solution to a MAP of 70 mm Hg; Hespan, Hespan infusion to a MAP of 70 mm Hg; Hextend, Hextend infusion to a MAP of 70 mm Hg; LR-BL, LR infusion to baseline MAP.

\* $p$   $<$  0.05, \*\* $p$   $<$  0.01 as compared to baseline; <sup>②</sup> $p$   $<$  0.05 as compared to the LR group.

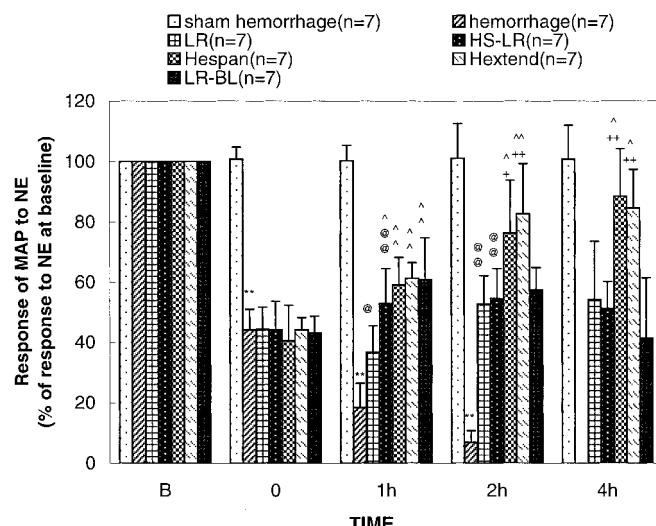
**Table 2** The Changes in MAP (mm Hg) after Hemorrhagic Shock in the Rat with or without Fluid Resuscitation

Group	Baseline	End of Hem (0 h)	Postinfusion		
			1 H	2 H	4 H
Sham hemorrhage (n = 7)	110.1 ± 7.97	111.8 ± 5.20	108.2 ± 5.51	109.4 ± 5.65	111.8 ± 4.79
Hemorrhage (n = 7)	112.4 ± 7.51	50.4 ± 0.33**	33.5 ± 7.72***#	17.5 ± 2.08***#(n=5)	
LR (n = 7)	108.4 ± 7.27	50.3 ± 0.58	69.9 ± 0.56	70.2 ± 2.04	69.6 ± 0.69
HS-LR (n = 7)	107.6 ± 7.53	50.5 ± 0.82	69.6 ± 0.89	69.6 ± 0.91	69.6 ± 1.06
Hespan (n = 7)	113.5 ± 6.63	50.2 ± 0.82	69.2 ± 0.88	70.2 ± 1.15	70.5 ± 1.19
Hextend (n = 7)	106.6 ± 6.69	50.5 ± 0.90	69.9 ± 0.84	70.6 ± 1.47	69.6 ± 1.06
LR-BL (n = 7)	109.7 ± 6.19	50.3 ± 1.01	108.6 ± 5.16	106.2 ± 6.82	106.5 ± 6.84

End of Hem, the end of the hypotensive period; LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg; HS-LR, 7.5% hypertonic saline infusion for 1 h followed by lactated Ringer's solution to a MAP of 70 mm Hg; Hespan, Hespan infusion to a MAP of 70 mm Hg; Hextend, Hextend infusion to a MAP of 70 mm Hg; LR-BL, LR infusion to baseline MAP.

\*\* p < 0.01 as compared to sham hemorrhage group; # p < 0.01 as compared to the end of hypotensive period (0 h).

mm Hg essentially maintained the vascular reactivity of these vasculatures at levels observed at the end of the hypotensive period. At 1 hour, the effect of HS infusion was better than LR, especially in the SMA and LRA, but this advantage was



**Fig. 3.** Effects of crystalloid and colloid resuscitation on the pressor response of MAP to NE (3  $\mu$ g/kg) after hemorrhagic shock in the rat. The increase in MAP after NE at baseline is considered a 100% response. B, baseline; Time 0, the end of the hypotensive period. n = 5 at 2 hours and n = 0 at 4 hours in the hemorrhage group; n = 7 at all other time points in each group. LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg for 4 hours; HS-LR, 7.5% hypertonic saline infusion for 1 hour followed by lactated Ringer's solution to a MAP of 70 mm Hg for 3 hours; Hespan, Hespan infusion to a MAP of 70 mm Hg for 4 hours; Hextend, Hextend infusion to a MAP of 70 mm Hg for 4 hours; LR-BL, LR infusion to baseline MAP for 4 hours. No shed blood was reinfused in any group. Data expressed as mean  $\pm$  SD. At time 0, all hemorrhage groups are significantly lower than the sham hemorrhage group. \*\*p < 0.01 as compared with the sham hemorrhage group; @p < 0.05, @@p < 0.01 as compared with the hemorrhage group; ^p < 0.05, ^^p < 0.01 as compared with the LR group; +p < 0.05, ++p < 0.01 as compared with the HS-LR group.

not maintained when LR was infused over the next 3 hours in the HS-LR group. Hespan and Hextend infusion significantly improved the impaired vascular reactivity in all four arteries (Fig. 4). In addition, their effects were significantly superior to both the LR and HS-LR groups in the SMA, LFA, and LRA. LR infusion to baseline MAP improved vascular reactivity in all arteries and was superior to LR infusion at 1 hour, but the effect was not maintained, and prolonged infusion deteriorated the vascular reactivity to NE (Fig. 4). The degree of recovery of vascular reactivity after infusion of the various fluids was different in these vasculatures. In general, vascular reactivity in the SMA and LRA in all infused groups was restored better than in the CA and LFA, especially in the Hespan and Hextend groups (Fig. 4) ( $p < 0.05$ ).

## DISCUSSION

Fluid resuscitation is an essential modality in the treatment of hemorrhagic shock. The quest for the best fluid for the resuscitation from hemorrhagic hypotension has led to a long-standing and continuing debate over the use of crystalloids or colloids and normotensive (aggressive) or hypotensive (limited) resuscitation. Despite this controversy, both types of fluids have been used clinically under certain circumstances. LR is often used as the standard of care.<sup>27</sup> HS has been shown to be an effective small-volume plasma volume expander<sup>11,12</sup> and has recently been recommended for use by an Institute of Medicine report.<sup>28</sup> Hextend was recently approved by the Food and Drug Administration for the treatment of hypovolemia after large-volume blood loss and Hespan is recommended for fluid resuscitation of hemorrhage for the U.S. Military Special Operations forces.<sup>14</sup> However, none of these fluids has been evaluated in vivo for their potential ameliorating effects on the vascular hyporesponsiveness observed after hemorrhage.

The present study indicated that 50 mm Hg hemorrhagic hypotension for 60 minutes without shed blood reinfusion caused significant loss of overall vascular reactivity to NE, as indicated from changes in MAP, in agreement with previous observations by ourselves<sup>7</sup> and others.<sup>8,24</sup> In addition, the observed decrease in the contractile response of blood vessels

**Table 3** Pre-NE Arterial Blood Flow (mL/min) after Hemorrhagic Shock and Crystalloid and Colloid Resuscitation

	Baseline	End of Hem (0 h)	Postinfusion		
			1 H	2 H	4 H
<b>SMA</b>					
Sham hemorrhage	4.68 ± 0.71	4.55 ± 0.77	4.51 ± 0.62	4.61 ± 0.77	4.87 ± 0.92
Hemorrhage	4.81 ± 0.67	1.54 ± 0.37**	0.61 ± 0.44**	0.18 ± 0.13**	
LR	4.72 ± 0.34	1.49 ± 0.28	5.13 ± 1.71@@	6.77 ± 2.57@@	7.56 ± 3.22
HS-LR	4.70 ± 1.29	1.47 ± 0.24	3.04 ± 1.07	4.21 ± 2.30	5.83 ± 3.06
Hespan	4.89 ± 0.35	1.45 ± 0.45	5.05 ± 1.09	5.55 ± 1.32	7.02 ± 2.69
Hextend	4.98 ± 1.05	1.47 ± 0.23	5.53 ± 1.81	7.44 ± 2.26	9.38 ± 1.43
LR-BL	4.83 ± 0.82	1.54 ± 0.40	11.38 ± 2.92^++**	10.2 ± 4.59++**	11.4 ± 4.59++**
<b>CA</b>					
Sham hemorrhage	3.76 ± 0.95	3.67 ± 1.04	3.58 ± 1.03	3.52 ± 1.09	3.51 ± 1.21
Hemorrhage	3.68 ± 0.17	0.73 ± 0.15**	0.22 ± 0.06**	0.13 ± 0.07**	
LR	3.58 ± 0.36	0.63 ± 0.25	1.99 ± 0.98@@	2.69 ± 1.35@@	3.17 ± 1.52
HS-LR	3.74 ± 0.34	0.73 ± 0.21	1.64 ± 0.36	2.46 ± 0.75@	2.98 ± 0.29
Hespan	3.96 ± 0.79	0.72 ± 0.36	2.00 ± 0.64	2.03 ± 0.67	2.45 ± 0.73
Hextend	3.77 ± 0.85	0.70 ± 0.25	2.29 ± 0.73	2.52 ± 0.82	2.89 ± 0.66
LR-BL	3.88 ± 0.66	0.78 ± 0.34	5.94 ± 2.41^++**	5.01 ± 1.83^++	4.82 ± 2.27
<b>LFA</b>					
Sham hemorrhage	1.11 ± 0.63	1.07 ± 0.44	1.09 ± 0.36	1.00 ± 0.38	1.11 ± 0.48
Hemorrhage	1.22 ± 0.31	0.43 ± 0.09**	0.22 ± 0.03**	0.13 ± 0.03**	
LR	1.09 ± 0.21	0.38 ± 0.08	0.82 ± 0.28@	0.85 ± 0.34@	0.78 ± 0.49
HS-LR	1.24 ± 0.34	0.41 ± 0.06	0.53 ± 0.20	0.59 ± 0.27	0.97 ± 0.48
Hespan	1.22 ± 0.46	0.34 ± 0.16	0.71 ± 0.24	0.91 ± 0.34	0.91 ± 0.33
Hextend	1.29 ± 0.52	0.42 ± 0.14	1.11 ± 0.62	1.47 ± 0.88	1.43 ± 0.90
LR-BL	1.21 ± 0.35	0.47 ± 0.16	2.60 ± 1.37^++**	1.94 ± 0.61^++**	1.63 ± 0.65
<b>LRA</b>					
Sham hemorrhage	2.26 ± 0.78	2.26 ± 0.72	2.16 ± 0.77	2.13 ± 0.79	2.24 ± 0.79
Hemorrhage	2.18 ± 0.16	0.46 ± 0.11**	0.16 ± 0.04**	0.07 ± 0.06**	
LR	2.22 ± 0.49	0.38 ± 0.13	1.06 ± 0.19@	1.11 ± 0.33@	0.98 ± 0.35
HS-LR	2.32 ± 0.87	0.47 ± 0.14	0.67 ± 0.33	0.91 ± 0.34	1.01 ± 0.44
Hespan	2.41 ± 0.74	0.40 ± 0.09	1.00 ± 0.34	1.35 ± 0.49	1.28 ± 0.58
Hextend	2.34 ± 0.58	0.43 ± 0.22	1.09 ± 0.59	1.43 ± 0.64	1.73 ± 0.61
LR-BL	2.29 ± 0.93	0.46 ± 0.18	2.72 ± 0.95^++	3.18 ± 1.02^++**	2.98 ± 1.18^++

End of Hem, the end of the hypotensive period. n = 5 at 2 h after hypotensive period in hemorrhage group, n = 7 at all other time points in each group. LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg; HS-LR, 7.5% hypertonic saline infusion for 1 h followed by lactated Ringer's solution to a MAP of 70 mm Hg; Hespan, Hespan infusion to a MAP of 70 mm Hg; Hextend, Hextend infusion to a MAP of 70 mm Hg; LR-BL, LR infusion to baseline MAP.

At time 0 h, blood flows in all arteries of hemorrhaged rats are significantly lower than blood flows in arteries of sham hemorrhaged rats. At 1 and 2 h, blood flows in all fluid resuscitation groups are significantly higher than blood flows in the hemorrhage only group.

\* < 0.05, \*\* p < 0.01 as compared to sham hemorrhage group.

@ p < 0.05, @@ p < 0.01 as compared to hemorrhage group.

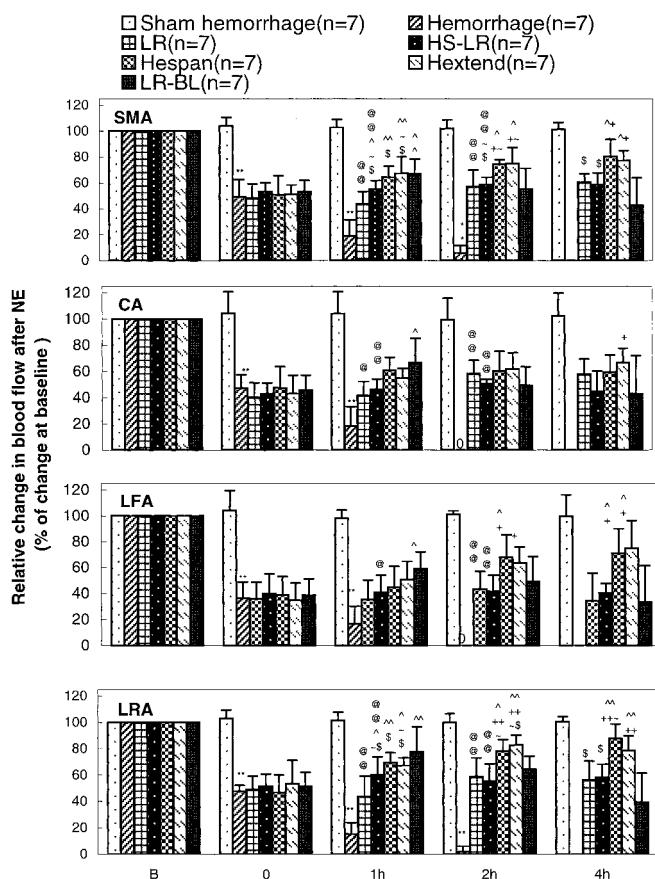
^p < 0.01 as compared to LR group.

++ p < 0.01 as compared to HS-LR group.

to NE, as determined by the change in blood flow in all four arteries evaluated, also agreed with our previous observations.<sup>7</sup> LR infusion to a MAP of 70 mm Hg prevented a further decrease in vascular reactivity induced by hemorrhage. HS infusion for the first hour followed by LR infusion significantly decreased the total volume of the fluid infusion and also improved the vascular reactivity to NE. Its improvement in the first hour was superior to LR alone. The beneficial effects of Hespan and Hextend were significantly better than LR or HS-LR. LR infusion to baseline MAP largely increased the fluid infusion volume and caused a marked hemodilution and blood flow increase in all observed arteries. Although it improved vascular reactivity better than LR at the 1-hour time point, prolonged infusion of large volumes of LR

deteriorated the vascular reactivity. Also, the improvement in vascular reactivity was not related to an improvement in blood flow in these vessels.

Hypertonic resuscitation fluids are known to be effective in restoring circulating volume in hypovolemia. The effect of 7.5% HS infusion in the present study probably results from its volume expansion, vasodilation, and anti-inflammatory effects.<sup>15,16</sup> This latter action may help explain the present observations, because some inflammatory cytokines or mediators such as endothelin, tumor necrosis factor- $\alpha$ , interleukin-1, complement, and oxygen-derived free radicals have been found to mediate the vascular hyporeactivity after trauma, shock, and sepsis.<sup>29-34</sup> Some studies have demonstrated that HS can decrease neutrophil (PMN) sequestration,



**Fig. 4.** Effects of crystalloid and colloid resuscitation on the blood flow responses of superior mesenteric artery (SMA), left renal artery (LRA), celiac artery (CA), and left femoral artery (LFA) to norepinephrine (NE) (3  $\mu$ g/kg, intravenously) after hemorrhagic shock in the rat. The relative change in blood flow after NE administration at baseline was taken as 100% response. B, baseline; Time 0, the end of the hypotensive period.  $n = 5$  at 2 hours and  $n = 0$  at 4 hours in the hemorrhage group;  $n = 7$  at all other time points in each group. LR, lactated Ringer's solution infusion to a MAP of 70 mm Hg for 4 hours; HS-LR, 7.5% hypertonic saline infusion for 1 hour followed by lactated Ringer's solution to a MAP of 70 mm Hg for 3 hours; Hespan, Hespan infusion to a MAP of 70 mm Hg for 4 hours; Hextend, Hextend infusion to a MAP of 70 mm Hg for 4 hours; LR-BL, LR infusion to baseline MAP for 4 hours. No shed blood was reinfused in any group. Data expressed as mean  $\pm$  SD. At time 0, all hemorrhage groups are significantly lower than the sham hemorrhage group. \*\* $p < 0.01$  as compared with the sham hemorrhage group; @ $p < 0.05$ , @@ $p < 0.01$  as compared with the hemorrhage group; ^ $p < 0.05$ , ^@ $p < 0.01$  as compared with the LR group; + $p < 0.05$ , ++ $p < 0.01$  as compared with the HS-LR group; ~ $p < 0.05$  as compared with the CA; \$ $p < 0.05$  as compared with the LFA.

adhesion, and activation, and reduce receptor linked up-regulation of adhesion molecules and the production of oxygen-derived free radicals and some cytokines.<sup>35,36</sup> For example, Rizoli et al. found that HS could reduce PMN sequestration in the lung and attenuate PMN-mediated tissue damage by pre-

venting lipopolysaccharide-mediated PMN CD11b expression after hemorrhagic shock in rats.<sup>37</sup> In addition, Ciesla et al. found that HS attenuated platelet activating factor priming of the PMN cytotoxic response by inhibition of p38 MAP kinase signaling.<sup>16</sup>

The appropriate volume of HS for resuscitating hypovolemic shock is estimated to be 4 to 6 mL/kg.<sup>38</sup> Our pilot study showed that continuous infusion of HS to maintain a MAP of 70 mm Hg for 2 hours resulted in the death of shocked rats, probably related to the total required infusion volumes of approximately 25 mL/kg. This may relate to the profound vasodilatory effects of HS on the vasculatures of the rat and resulting hypernatremia. The volume of infusion of HS to maintain a MAP of 70 mm Hg for 1 hour was equivalent to 5.1 mL/kg. Thus, infusion of HS to maintain a MAP of 70 mm Hg for 1 hour, followed by LR infusion for 3 hours, was adopted in this experiment in the HS-LR group, and this regimen resulted in only a slight increase in plasma sodium concentrations at 4 hours. At present, it is unknown whether the limitation of HS use observed in the current study is unique for the rat or whether it would extend to other species.

Colloidal solutions, especially those solutions containing hydroxyethyl starch macromolecules such as Hextend and Hespan, have been reported to attenuate ischemia-reperfusion injury of some tissues and organs.<sup>13,39,40</sup> Hydroxyethyl starch-mediated tissue protection may include a capillary "sealant" effect that prevents tissue edema,<sup>39</sup> reduced PMN adherence to endothelial cells that alleviate PMN-mediated damage,<sup>41</sup> and an antioxidant effect.<sup>13</sup> Nielsen et al.<sup>13</sup> reported Hextend resuscitation significantly decreased the multiple organ injury and systemic injury associated with hepatoenteric ischemia-reperfusion in rabbits and significantly reduced the release of xanthine oxidase activity (approximately 50%) as compared with albumin or LR. They speculated that the decreased organ injury after hetastarch administration was closely related to its antioxidant activity. In addition, Tait and Larson<sup>17</sup> reported Hespan resuscitation after hemorrhagic shock resulted in a compensatory organ hyperemia and tissue blood flow improvement, and a significant increase in oxygen transport. In contrast, resuscitation with LR produced significant hemodilution without hyperemia and, consequently, a significant decrease in oxygen transport. The present study found that Hespan or Hextend resuscitation required the least volume, resulted in lower hemodilution, and produced similar or better blood flow in the observed arteries as compared with LR infusion. Taken together, these mechanisms may help explain how Hextend and Hespan improved vascular reactivity superior to LR in this study, but further evaluation is warranted.

Traditional fluid resuscitation to normalize the blood pressure rapidly is being challenged, especially for treating hemorrhagic shock victims with penetrating injuries.<sup>20,27</sup> It has been argued that normotensive resuscitation can increase bleeding and worsen outcome because of severe hemodilution and disruption of newly forming blood clots. The con-

cept of permissive hypotensive resuscitation has been introduced to avoid these consequences.<sup>20,27,42,43</sup> The present study compared the effects of LR infusion to a MAP of 70 mm Hg or to the baseline level. The results indicated that hypotensive resuscitation with LR prevented the further decrease of vascular reactivity induced by hemorrhage. Normotensive resuscitation with LR improved vascular reactivity only for a short time, and prolonged infusion did not improve hemorrhagic shock-induced vascular hyporesponsiveness, but worsened it. The Hct in this group was decreased to 14.9%, which was significantly lower than the Hct of 21.6% in the LR group. Previous studies reported that during isovolemic hemodilution, Hct as low as 14% would obviously reduce oxygen delivery.<sup>44</sup> This may cause severe ischemia and hypoxia of tissue and result in tissue/cell ischemic injury including vascular endothelial cell damage.<sup>45</sup> In addition, resuscitation with a large volume of LR can activate complement and PMN, which can alter vascular reactivity.<sup>15,29</sup>

The present study and our previous observation<sup>7</sup> found that the loss of vascular reactivity after hemorrhagic shock varied among different vascular beds. These observations may be related to the diversity of the expression and secretion of some signaling molecules and cytokines such as nitric oxide, endothelin, tumor necrosis factor- $\alpha$ , and interleukin-1 in the organs, and the different rates of perfusion in these organs.<sup>8,46–49</sup> Ischemia and hypoxia of tissues can induce the production of oxygen free radicals and tissue acidosis, which also play important roles in the occurrence of vascular hyporeactivity after shock.<sup>34,50</sup> In the current study, recovery of vascular reactivity after infusion of the various fluids differed somewhat among the four vasculatures examined. Generally, vascular reactivity in the SMA and LRA in all fluid-infused groups was restored better than in the CA and LFA. The actual mechanisms responsible for this differential restoration of vascular reactivity in different vascular beds observed remain unknown and warrant further study.

Two areas of future research in this area could focus on gender effects and the effects of progressive acidosis on the actions of NE. Numerous studies in experimental animals have reported that male gender is associated with immunosuppression and worsened outcome in response to trauma and hemorrhage.<sup>51</sup> In addition, testosterone could reduce vascular relaxation induced by the testosterone receptor antagonist flutamide.<sup>52</sup> Since the present study used male rats, it is unknown whether similar results would be observed in female rats. In the present study, arterial pH was not monitored throughout the study. Therefore, it is unknown whether this model was associated with an acidosis that could influence the action of NE. Further work is required to investigate the complex interactions among pH, catecholamine activity and vascular smooth muscle cell function.

It should also be mentioned that in the present study, we have referred to changes in blood flow in response to NE in the individual arteries as changes in vascular responsiveness. This implies changes in vascular tone. However, it could be

argued that effects of NE independent of vascular tone could be influencing the present results. For example, NE can have direct effects on the heart and increase stroke volume, which can lead to pressure-driven blood flow changes. However, in the current study, blood flow decreased after NE infusion. Also, because NE has minimal  $\beta_2$ -adrenergic receptor agonist activity, it is unlikely that the decreased blood flow observed is caused by vasodilation. Nevertheless, we acknowledge that our data only indirectly reflect vascular reactivity.

In conclusion, 50 mm Hg hemorrhagic hypotension for 60 minutes without shed blood reinfusion caused an apparent loss of overall (as determined by MAP) and individual vascular reactivity to NE. Hypotensive resuscitation with LR alone or combined with HS slightly improved the loss of vascular reactivity after hemorrhagic shock. Of the fluids examined, colloids such as Hespan and Hextend infusion had the most beneficial effects on improving vascular reactivity compared with LR or HS plus LR and required the least volume to maintain MAP at 70 mm Hg. Prolonged normotensive infusion with LR resulted in severe hemodilution and deterioration of vascular reactivity. The four vasculatures examined had slightly different responses to the infusion of various fluids such that the recovery of reduced vascular reactivity in the SMA and LRA was somewhat better than in the CA and LFA. Further studies are required to determine the mechanism behind this improvement in various vascular beds and to determine whether these effects are important in improving outcome.

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